Tension maintenance and crossbridge detachment

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Received 21 May 1982

1. INTRODUCTION

Glycerol-extracted insect flight muscles held under tension at constant length in the absence of nucleotide (rigor) will maintain that tension overnight [1]. The time of dissociation of heavy meromyosin from actin under similar ionic conditions is ~20 min [2]. Therefore, crossbridges in the filament lattice may be hindered from detachment by the presence of their neighbours [1]. We have explored this idea further by repeating the observations [1] on tension maintenance in the presence of an unhydrolysed nucleotide (adenylylimidodiphosphate: AMPPNP) which enhances the dissociation rate of acto-heavy meromyosin in vitro [2, 3]. We find that tension is still maintained overnight, as if either stabilising effect of the lattice were great or that force has a large effect on crossbridge kinetics. Tension is maintained in both glycerol-extracted insect flight and rabbit psoas muscle fibres, so stabilisation does not appear to depend upon a particular geometrical arrangement of the filaments.

2. MATERIALS AND METHODS

AMPPNP (1 mM, Sigma) was used in combination with either 10 or 100 μM of the myokinase inhibitor diadenosine pentaphosphate (AP_5A) (kindly provided by Dr R.S. Goody) and 1 mM glucose + 0.1 mg hexokinase/ml (Sigma) to eliminate residual ATP. Rabbit psoas muscle fibre bundles were prepared by glycerol extraction combined with osmotic shock treatment [4]; *Lethocerus* dorsal longitudinal flight muscles were glycerol-extracted without osmotic shock [5]. Both tissues were held at − 20°C for 2–8 weeks in 50% glycerol before use.

A single fibre of either muscle was mounted under tension in the apparatus of [5]; insect fibres were used near to rest sarcomere length (2.3 μm) and rabbit fibres at 2.5–2.8 μm. They were incubated in a series of solutions at 7°C (pH 7.0). These solutions contained either 50 mM (ionic strength = 0.09) or 110 mM (ionic strength = 0.145) KCl, 5 mM Pipes, 6 mM $MgCl_2$, 5 mM EGTA and 1 mM NaN_3. In nucleotide solutions the [KCl] was adjusted to maintain constant ionic strength. The tension was continuously analogue-recorded and the muscle stiffness estimated at intervals by the tension excursion in response to a 5 or 105 Hz sinusoidal length oscillation of 0.05%. After a first wash in the nucleotide-free salt solution (rigor) to remove glycerol the muscle fibre was relaxed by addition of 5 mM ATP. This nucleotide was then washed out and AP_5A, glucose and hexokinase introduced into the rigor buffer solution. After 20 min AMPPNP was added and the fibre was extended by 1%. It was then held at constant length for 15–17 h. Finally, it was cycled through ATP and base solution to test its ability to relax and develop rigor tension.

The microscopic appearance of rabbit muscle fibres held under similar conditions was examined by suspending bundles of 4–6 fibres between supports on a cavity slide and observing them under phase contrast. The fibres were incubated overnight in AMPPNP at 4°C or 16°C and changes in their appearance sought.

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Published by Elsevier Biomedical Press
001457593/82/0000–0000/$2.75 © 1982 Federation of European Biochemical Societies

3. RESULTS

Four experiments were performed on rabbit muscle fibres at 7°C, ionic strength = 0.09 and 10 μM AP_5A. On adding ATP the tension in the muscle fibre fell to zero. On its removal it rose quickly to reach 250–350 μN. On addition of AMPPNP it fell to 50–75% of its value in rigor [5]. On extension of the muscle it rose to close to the rigor value (270–300 μN). Whilst held isometric, the tension then fell, rapidly at first. Within 100 min it appeared stable at 160–170 μN. It did not perceptibly alter overnight (fig.1); for 100–1000 min. $\Delta T = +2$ to -3 μN (5% fig.1). The 5 Hz stiffness also remained constant at 22–27 mN, between fibres. At the end of the experiments, the fibres still relaxed and pulled a high rigor tension, 210–300 μN (fig.1).

Two experiments were performed on insect fibres under the same chemical conditions except that 100 μM AP_5A was used. Again, a stable tension (180, 150 μN) was obtained within 100 min and showed no perceptible decrease overnight.

Three further experiments were performed on rabbit fibres at a higher temperature and ionic strength (10°C, ionic strength = 0.145, 100 μM AP_5A); 2 h after extension the tension stabilised at 110– 204 μN and no further reduction could be seen overnight, nor did the stiffness (measured at 105 Hz) alter.

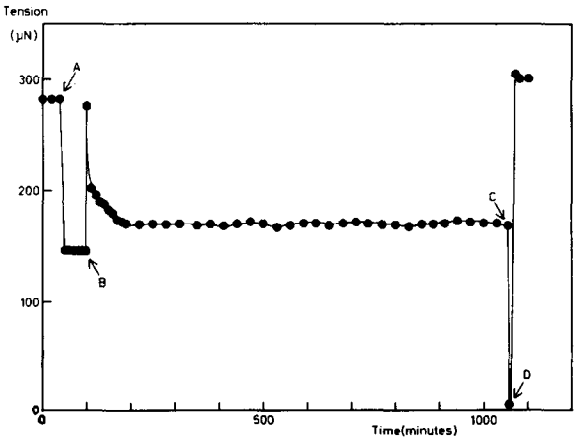

Fig.1. Tension in a single glycerol-extracted rabbit psoas muscle fibre held isometric except at point (B): at (A) 1 mM AMPPNP was added; at (B) the fibre was stretched by 1%; at (C) the AMPPNP was removed and 5 mM ATP added; at (D) the ATP was removed.

The regularity of the sarcomeres in 7 rabbit fibre bundles stretched over cavity slides had not changed after overnight incubation in AMPPNP, and 2 slack bundles did not straighten out.

4. DISCUSSION

AMPPNP is not hydrolysed by myosin [6] but ADP, which is present as a small percentage contaminant in AMPPNP preparations, could be rephosphorylated by the endogenous myokinase and then hydrolysed by the muscle fibre myosin. The conditions that we chose were designed to avoid this:

(i) By competition of AP_5A with contaminant ADP for myokinase [7];
(ii) By competition of hexokinase with myosin for any ATP formed.

It is probable that these precautions were adequate, since the results were insensitive to [AP_5A], and during prolonged AMPPNP incubation fibres showed no signs of contraction.

Fibres reached the steady tension level seen here after 2 phases of tension fall: (1) after AMPPNP was added; and (2) after the fibre had been extended. Phase (1) is rapid and reversible and could be due to reorientation of attached crossbridges on binding of the nucleotide [5]; phase (2) is slow and irreversible, and probably represents detachment of overstressed crossbridges [1].

Once the tension stabilised it remained constant for the duration of the experiment: the maximum rate-constant for an assumed first-order tension decay consistent with our results from rabbit fibres was some $3 \times 10^{-7}\ s^{-1}$ (< 2% fall in 900 min).

This result may be contrasted with the relative ease with which myosin heads detach from actin filaments in vitro; in the presence of AMPPNP the acto-Sl detachment rate at pH 8, 16°C is $16\ s^{-1}$ [3] while that of acto-HMM at pH 7, 25°C is $1.7\ s^{-1}$ [2]. The kinetics of crossbridges in the muscle fibre under tension and when in solution are thus different, and the long tension maintenance requires explanation.

Kuhn [1] supposed that crossbridge detachment is hindered by the presence of neighbouring crossbridges. If this is the case, these results show that the effect is large, reducing the detachment rate by a factor of at least 10^6. It also appears to be independent of the particular crossbridge grouping induced

by the filament lattice, since AMPPNP alters the grouping [8, 9]. If a similar effect occurs in active muscle, it will have to be considered in relating protein kinetics to crossbridge kinetics.

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